37. (Amended) The method of claim 36, further comprising the step of expressing a second exogenous polypeptide in said at least a portion of said cells, said second exogenous polypeptide being capable of forming functional gap junctions within said at least a portion of said cells.

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-37 are in this case. Claims 1-22 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 23-37 have been rejected. Claims 23 and 37 have now been amended.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 23-37 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, has possession of the claimed invention. The Examiner's rejections are respectfully traversed. Claims 23 and 37 have now been amended.

The Examiner points out that the specification does not assert any use, other than treatment, for the claimed method of cellular implantation.

The present invention relates to methodology, which can be used to modify the electrophysiological function of cells or tissues of, for example, damaged myocardium, neurons and secretory glands.

Although one objective of the present invention is indeed restoration of normal electrophysiological function to damaged tissues such as heart, nerve or glandular tissues and thus treatment of a variety of disorders associated with such tissues, one of ordinary skill in the art privileged to the teachings of the instant application, would instantly recognize that the present methodology can also be utilized to elucidate the normal physiology of excitable tissues as well

as for studying and uncovering therapeutic agents that can modify the electrophysiological function of excitable tissues.

For example, excitable tissue cultures and animal models modified according to the present methodology can be used to identify molecules having a desirable therapeutic effect. Specifically, tissue culture models constructed with accordance to the guidelines provided in the instant application can be utilized in assays designed for toxicological investigations of cardio-active drugs. Furthermore, compounds for use in therapy can be tested in suitable animal model systems (e.g., rats, cows, mice, monkeys, rabbits) and the like prior to testing in humans.

Thus, in view of the fact that such applications and others would be readily apparent to one of ordinary skill in the art privileged to the teachings of the instant application, Applicant is of the opinion that the specification asserts several uses for the claimed method of cellular implantation.

The Examiner further points out that the teachings of the specification are limited to analysis of conduction properties of cells in cultures in a variety of assays and that the claims are broad in scope encompassing a wide variety of diseases and disorders.

As is clearly illustrated in the instant application and in the attached Appendix, which presents results obtained following filing of the instant application, using the teachings of the instant application, the present inventors demonstrate, for the first time, modification of electrophysiological properties of myocardial tissues.

Applicant wishes to point out that since the method of the present invention is clearly capable of regulating electrophysiological properties of such a tissue, the electrophysiological function of any excitable tissue can in effect be regulated by using the present methodology.

As described in the instant application, it is well known that tissue excitability is governed by ion channels. Thus, for example, myocardial contraction depends on the opening and closing of a complex series of ion channels in myocardial cell membranes. Signal propagation through neuronal

cells is also governed by ion influx/outflux through nerve cell membranes. In nerve cells, sodium, calcium and potassium channels participate in the generation and propagation of a nerve signal. Secretion from glandular tissues also depends on ion channels. For example, in the pancreas, T-type calcium channels and cell-to-cell gap junctions participate in insulin secretion (see the Background section of the instant application, Pages 7-8 lines 11-12). Another example, is the ion channel-regulated fluid secretion from the salivary glands. Such secretion is activated by an increase in cytosolic calcium which regulates a number of ion transporters, e.g., Ca^{2+}-activated K^+ channel, $Na^+/K^+/2Cl^-$ co-transporter in the baso-lateral membrane, and the Ca^{2+}-activated Cl^- channel in the luminal membrane, which are intricately involved in fluid secretion [Ambudkar (2000) Crit. Rev. Oral. Biol. Med. 11:4-25].

The central role of ion channels in excitable tissue function is further substantiated by the pathophysiology of a number of channel mutations with inherited paroxysmal and chronic disorders such as episodic ataxia with myokymia and ventricular arrhythmia with Q-T prolongation.

Furthermore, an increasing number of epileptic syndromes is associated with mutations in ion channels; Benign familial neonatal convulsions are caused by mutations in potassium channels constituting the M-current (KCNQ2, KCNQ3), generalized epilepsy with febrile seizures plus is caused by mutations in subunits of the voltage-gated sodium channel or the GABA(A) receptor (SCN1B, SCN1A, GABRG2), and episodic ataxia type 1 is associated with mutations within another voltage-gated potassium channel [KCNA1, Lerche (2001) AM. J. Med. Genet. 106:146-59].

The use of ion channel blockers (further described hereinbelow) in the treatment of a wide range of diseases associated with impaired function of excitable tissues such as diabetes and epilepsy further substantiates the role of ion channels in excitable tissue functionality and validates the use of the present methodology for the treatment of a wide range of diseases in a wide range of tissue types.

Thus, given the central and well established role of ion channels in tissue excitability and given the fact that application of the present methodology to one complex tissue type (i.e., cardiac tissue) conclusively proved that modification of the electrophysiological property of such tissue is possible, it is applicant's strong opinion that application of the present invention to any tissue is clearly enabled by the teachings of the instant application.

The ability to change the electrophysiological properties of any excitable tissue offers the opportunity to treat a wide range of diseases or disorders such as for example, which could only be otherwise treated by organ transplantation, a procedure plagued by numerous limitations.

The present methodology can also be used to treat a variety of inherited disorders associated with hyper- or hypoexcitability of tissues, such as channelopathies. Channelopathies include a number of human diseases, such as ventricular tachycardia, hypertension, deafness and certain metabolic disorders, which are associated with ion channel dysfunction.

Although some of the above described diseases can be treated with drugs which specifically block or activate individual subtypes of ion channels, [e.g., the anti-diabetic glibenclamide (K channel blocker) and the anti-hypertensive and anti-arrhythmic nifedipine (Ca channel blocker)], such drugs have limited clinical use due to severe adverse effects.

In sharp contrast, the present methodology allows to modify electrophysiological properties of excitable tissues in a localized manner, thus avoiding the adverse cytotoxicity, which is associated with pharmacological treatments and promoting therapeutic efficacy.

Thus, it is Applicant strong opinion that any disorder associated with altered electrophysiological properties of excitable tissues can be treated using the method of the present invention.

Applicant further wishes to point out that there is a direct relation between conductivity and tissue excitability and thus conductivity is a good

measure of tissue functionality; the contributing roles of ion channels to the generation of electrical signaling are well documented.

For example, excitability and conductivity underlie the electrophysiological properties of the cardiac tissue. It has been observed that alteration or impairment of these interrelated properties may result in cardiac arrhythmias. Such disorders are commonly treated by artificial cardiac pacing, by which rhythmic electrical discharges are applied to the heart at a desired rate from an implanted artificial pacemaker. The pulses delivered to the heart for pacing therapy need only be of sufficient magnitude to stimulate the excitable myocardial tissue in the immediate vicinity of such pacing electrode.

Conductivity is also a good measure of glandular tissue function. For example, it is well known that insulin-secreting pancreatic beta cell are electrically excitable and changes in the membrane potential play an important role in coupling the metabolism of glucose to the discharge of the insulin-containing granule. Accordingly, the application of the patch-clamp technique (enables recordings of currents associated with the opening of individual ion channels) to pancreatic islet cells has revolutionized understanding of the beta cell electrophysiology.

Although most of the assays presented in the instant application illustrate electrical conductivity of modified tissues, the present inventors were also able to show that cells modified according to the present methodology integrated well with the cells of the target tissue and exhibited long-term viability following implantation.

As is shown in Example 5 of the instant application and in the Appendix section enclosed with this response, several methods were used to characterize fibroblast survival and structural integration within the co-cultures. These included vital staining with Fast-DiO throughout experiments, and immunostaining with anti Kv1.3 at the end of the experiments showing the stable expression of the channel in transfected fibroblasts. Finally, co-staining with anti-MHC and anti Kv1.3 antibodies ruled out the possibility of

endogenous expression of Kv1.3 in cardiomyocytes by showing the absence of Kv1.3 staining in these cells.

The Examiner further points out that the specification does not provide working examples with regard to treatment of a diseased animal by cellular implantation.

The instant application teaches the ability of transfected fibroblasts to electrically couple with cardiac myocytes *in-vitro*, causing a significant local and reversible modification of electrophysiological properties thereof (see Example 5 of the instant application).

As shown in Example 5 of the instant application and in section I of the attached Appendix, in-vitro hybrid cultures generated according to the teachings of the present invention support electrical coupling between ion channel expressing fibroblasts and cardiomyocytes. Kv1.3 transfected fibroblasts caused significant changes in the electrophysiological properties of the hybrid cultures as manifested by significant reduction in extracellular signal amplitude and by the generation of multiple conduction blocks (Figures 5a-g and 6a-g of the attached Appendix). The location of all conduction blocks correlated with the spatial distribution of the transfected fibroblasts as determined by staining (Figures 5e-g of the attached Appendix). All electrophysiological changes were reversed following the application of Charbdotoxin, a specific Kv 1.3 blocker (Figure 5 of the instant application and Figures 5a-d of the attached Appendix). In contrast, conduction remained uniform in control hybrid cultures when NIH 3T3 without transfection fibroblasts were used (Figures 4a-d of the attached Appendix).

The marked electrophysiological changes could be exclusively attributed to the expression of Kv1.3 since NIH 3T3 without transfection fibroblasts could not mediate such an effect. Furthermore, application of the specific Kv1.3 blocker partially or completely reversed the effects of Kv1.3 expressing fibroblasts in a dose dependent manner.

Based on these *in-vitro* results it was anticipated that ion channel expressing fibroblasts may also alter the electrophysiological properties of an excitable tissue in vivo.

Indeed, results obtained following filing of the instant application (described in Section II of the Appendix enclosed herewith), illustrate the ability of genetically modified fibroblasts to modify the ventricular response of a rat's heart *in-vivo*. These results substantiate the ability of the cellular grafts generated according to the teachings of the present invention to alter the electrical properties of excitable tissues in-vivo.

As shown in Figure 8 of the Appendix, heart transplanted Kv1.3 expressing fibroblasts integrated well with the cardiac tissue and survived following transplantation. Furthermore, such cellular grafts could extend the refractory period in the left ventricular wall and AV node of transplanted rat hearts. In accordance with the in-vitro results, these effects were Kv1.3 mediated since wild type fibroblasts could not mediate such effects and a specific Kv1.3 inhibitor reversed the effects of the ion channel.

As is suggested in the instant application (e.g., Page 38-42 lines 17-4), the ability of Kv1.3 expressing fibroblasts to extend the refractory period can be used to treat various medical conditions such as atrial fibrillation (AF), wherein the normal rhythmical contractions of the cardiac atria are replaced by rapid irregular twitching of the muscular wall and the ventricles respond in an irregular and rapid manner to the dysrhythmic bombardment from the atria.

In view of the *in-vivo* results, and further in view of the strong correlation between these results and the *in-vitro* results presented in the instant application, Applicant strongly believes that the instant application provides one of ordinary skill in the art with the guidelines necessary to modify the electrophysiological properties of an excitable tissue, be it healthy or diseased.

Thus, Applicant is of the strong opinion that the instant application provides sufficient enablement for practicing the present invention on any excitable tissue and any disease.

Examiner further points out that the specification fails to provide an enabling disclosure for producing a therapeutic effect using the claimed method because of the unpredictability of methods of cellular transplantation and gene therapy.

Although difficulties in utilizing gene transfer technology as well as numerous other widely utilized molecular techniques have been reported in the art, successful implementation of these so called "unpredictable" techniques has been reported in numerous publications including patent publications which disclose treatment methods utilizing gene therapy.

In many cases, the unpredictability of an art lies not with the technique but rather with other factors, which at times are either unknown to the scientist or are uncontrollable thereby.

For example, chemotherapy is a widely used pharmacological technique for treating cancer. The effects of chemotherapy on cancer treatment cannot be predicted since it is influenced by parameters associated with the patient and/or the disease. As a result of its unpredictability, many of the patients treated do not actually benefit from chemotherapy, a fact which has not led to dismissal of this technique.

The present invention can be effected using direct DNA administration or transplantation of a population of cells which was ex-vivo transformed with the DNA (as shown in Section II of the Appendix attached herewith).

Successful implementation of both gene and cell therapy has been demonstrated by numerous published prior art studies. By May 2001, 532 gene therapy protocols have been approved for evaluation in clinical trials [Stephan et al. (2002) Oncologist 7(1):46-59].

Numerous ongoing studies involve gene therapy for treating various forms of human cancer.

Clinical Trials

Sterman et al. [(Hum. Gene Ther. 9:1083-92)] conducted a phase I trial of adenovirus mediated intrapleural Herpes-simplex virus (HSV)-thymidine kinase (tk)/ ganciclovir (GCV) gene therapy in patients with mesothelioma. A

replication-incompetent adenoviral vector containing the HSV-tk gene under control of the Rous sarcoma virus promoter-enhancer was introduced into the pleural cavity of patients with malignant mesothelioma followed by 2 weeks of systemic therapy with GCV at a dose of 5 mg/kg twice daily. Side-effects were minimal and included fever, anemia, transient liver enzyme elevations, and bullous skin eruptions as well as a temporary systemic inflammatory response in those receiving the highest dose.

This study demonstrated that intrapleural administration of an adenoviral vector containing the HSV-tk gene leads to detectable gene transfer when delivered at high doses as well as being tolerated by treated individuals.

Klatzmann et al. [Hum. Gene Ther. 9:2595-2604] used an HSV-tk/GCV system in which allogeneic M11 cells were transduced by retrovirus in vitro and injected into the surgical cavity (sc) after debulking of glioblastoma. Despite extensive surgery for glioblastoma, residual tumor cells always lead to relapse. After a 7-day transduction period, GCV was administered for 14 days. Twelve patients with recurrent glioblastoma were treated without serious adverse side effects. Twenty-five percent of the patients survived longer than 12 months. Four months following treatment, 4 of the 12 patients treated had no recurrence. One patient was still free of detectable recurrence, steroid free and independent, 32 months following treatment. Thus, injections of M11 retroviral vector cells producing tk resulted in significant therapeutic responses as well as being well tolerated by the treated individuals.

Stewart et al. [(1999) Gene Ther. 6:350-363] conducted a phase I study in which an E1- and E3-deleted adenovirus encoding IL-2 (Ad-CA-IL-2) was directly injected into sc deposits of melanoma and breast cancer. Local inflammation was observed at the site of injection in 60% of the patients, however, no severe side-effects were reported. Incomplete local tumor regression occurred at the site of injection in 24% of the patients. No Ad5E1 sequences were detected either prior to, or following injection, indicating the absence of replication-competent virus. Anti-adenovirus and neutralizing antibody titers were elevated 1 month following injection in all patients.

This trial, confirmed the safety of use of adenoviral vectors for gene delivery in humans and demonstrates successful transgene expression even in the face of preexisting immunity to adenovirus.

Palmer et al. [Hum. Gene Ther. 10:1261-8] used a recombinant retrovirus to transduce expression of IL-2 in melanoma cells. These IL-2-secreting tumor cells were then used to vaccinate individuals. Twelve patients were vaccinated sc 1, 2, or 3 times with approximately 10^7 irradiated, autologous, IL-2-secreting tumor cells. Treatment was well tolerated, with local reactions at 11 of 24 injection sites and minor systemic symptoms of fever and headache following 6 injections. One patient developed antitumor delayed-type hypersensitivity after the first vaccination and showed an increased response after the second vaccination. This study illustrated that vaccination with autologous, genetically engineered tumor cells is both feasible and safe and effective at inducing antitumor delayed-type hypersensitivity and anti-tumor cytotoxic T lymphocytes.

Herman et al. [(1999) Hum. Gene Ther. 10:1239-1249] also studied direct in situ gene therapy for adenocarcinoma of the prostate using a replication- deficient adenovirus expressing tk and administration of GCV. Patients received injections of increasing concentrations of virus into the prostate under ultrasound guidance. GCV was then given intravenously (iv) for 14 days (5 mg/kg every 12 hours). Eighteen patients received 1 x 10^8 to 1 x 10^{11} IU. All cultures of blood and urine specimens were negative for growth of adenovirus. One patient at the highest dose level developed spontaneously reversible thrombocytopenia and hepatotoxicity. Three patients achieved an objective response, documented by a fall in serum PSA levels by 50% or more, that was sustained for 6 weeks to 12 months.

Clearly gene therapy can be applied safely and effectively to human tumors by injection into pleural or tumor cavities or by direct tumor injection. The dosage of virus indicated in these trials caused at most mild side-effects.

Oncolytic virotherapy using adenovirus dl1520 (Onyx-015) – dl1520 (Pfizer Corp. Groton, CT, USA), an E1B – 55kD gene deleted adenovirus was

the first genetically engineered agent to be thoroughly tested in humans. The agent is an adenovirus modified to replicate in, and selectively kill, cells that harbor p53 mutations. Over 250 cancer patients have so far been treated in approximately 10 clinical trials (phase I-III). These studies showed that this modified virus is well tolerated by patients following intratumoral, intraperitoneal, hepatic arterial and iv administration. Viral replication was tumor selective and was documented following administration by all routes. Although single agent efficacy was limited, co-administration with chemotherapy exhibited anti-tumoral activity. These clinical research results demonstrated the potential of this novel treatment platform [Kirn (2001) Expert Opin. Biol. Ther. 1:525-38]. It will be appreciated that Onyx Pharmaceuticals was granted U.S. Pat. No. 5,677,178 covering methods for the treatment of p53 related cancers in 1997. The patent specifically covers the use of modified adenoviruses and other DNA viruses which lack viral proteins that bind to p53, for the treatment of cancer patients whose tumors lack p53 function. Other patents include U.S. Pat. No. 5,846,945 and EP Pat. No. 09491077.8 [Cohen (2001) Curr. Opin. Investig. Drugs 2:1770-5].

Shalev et al. [(2000) J. Urol. 163:1747-50] reviewed a case in which 52 patients were repeatedly injected with an adenovirus-expressing tk. Although toxicity increased from 35% up to 75%, in patients who received from 2–4 cycles of therapy, all toxic events were mild and resolved completely. No additional toxicity was noted. Results obtained from 28 patients indicated a mean decrease of 44% in PSA in 43% of the patients, showing that direct injection of HSV-tk, followed by iv GCV, was safe and effective, even in multiple trials.

Other examples of active or completed clinical trials are listed hereinbelow:

(i) Gene therapy of malignant gliomas: a Phase 1 study of IL-4-HSV-TK gene-modified autologous tumor to elicit an immune response. 1998 Active Principal Investigators: M. Bozik, H. Okada, M. Lotze; Collaborator: J. Barranger.

(ii) Gene therapy of melanoma, Phase 1, University of Michigan, Ann Arbor, MI 1992 Completed; Principal Investigator: G. Nabel; Collaborator: L. Huang.

(iii) Gene therapy of gynecological cancers, Phase 1, Singapore General Hospital, Singapore 1996 Completed; Principal Investigator: K. Hui; Collaborator: L. Huang.

(iv) Gene therapy for c-erB-2 overexpressing ovarian and breast cancers, Phase 1. 1996 Active; Principal Investigators: D. Hortobagyi, M.-C. Hung; Collaborator: L. Huang.

(v) A Phase II, multicenter open label study to evaluate effectiveness and safety of two treatment regimens of Ad5CMV-p53 administered by intra-tumoral injections in 78 patients with recurrent squamous cell carcinoma of the head and neck. 1997 Active; Principal Investigator: S. Agarwala; Collaborator: J. Barranger.

(vi) A Phase 1 study in patients with recurrent or metastatic squamous cell carcinoma of the head and neck using SCH 58500 (rAd/p53) administered by single intratumoral injection. 1996 Active; Principal Investigator: S. Agarwala; Collaborator: J. Barranger .

(vii) IL-12 gene therapy of melanoma using direct injection of tumors with genetically engineered autologous fibroblasts (Phase II study) 1996 Active; Principal Investigator: H. Tahara; Collaborator: J. Barranger.

(viii) IL-12 gene therapy for head and neck cancer melanoma using direct injection of tumors with genetically engineered autologous fibroblasts (Phase II study) 1996 Active; Principal Investigator: H. Tahara; Collaborator: J. Barranger.

(ix) IL-12 gene therapy using direct injection of tumors with genetically engineered autologous fibroblasts. 1996 Active; Principal Investigators: H. Tahara, M. Lotze; Collaborator: J. Barranger.

(x) Phase 1 study of percutaneous injections of wild-type adeno-virus p53 construct (Adeno-p53) for hepatocellular carcinoma. 1997 Active; Principal Investigators: C. Belani, C. Carr; Collaborator: J. Barranger.

Clinical trial of gene therapy for Gaucher disease. 1996 Active; Principal Investigator: J. Barranger.

(xi) Gene therapy for Canavan's disease, Phase I/II, University of Aukland, New Zealand. 1994 Active; Principal Investigators: M. During, P. Leone (Liposomes were produced in HGTAL by L. Huang).

(xii) Gene therapy for cystic fibrosis, Phase I, National Lung and Blood Institute, London, UK; 1995 Completed; Principal Investigators: E. Alton, D. Geddes, B. Williamson.

(xiii) Gene therapy for cystic fibrosis, Phase I, University of Oxford, Oxford, UK 1996 Completed; Principal Investigators: S. Hyde, D. Gill, C. Higgins.

(xiv) Gene therapy for cystic fibrosis by multiple dosing, Phase I, University of Oxford, Oxford, UK 1997 Completed; Principal Investigators: D. Gill, S. Hyde, C. Higgins (Liposomes were produced in HGTAL by L. Huang).

Delivery

Effective delivery of a virus into sites of expression has been demonstrated by numerous studies. Of particular interest is an approach which utilizes computer-aided tomography (CAT) to direct needle injection into a tumor. Such a technique has been demonstrated in the treatment of non-small cell lung cancer by Kauczor et al. [(1999) Eur. Radiol. 9:292-296]. In a prospective clinical phase I trial, six patients with non-small cell lung cancer and a mutation of the tumor suppressor gene p53 were treated by CAT-guided intratumoral gene therapy. Ten milliliters of a vector solution (replication-defective adenovirus expressing wild-type p53 cDNA) were injected under CAT guidance. The CAT-guided gene therapy was easily performed in all six patients without intervention-related complications. Besides flu-like symptoms, no significant adverse effects of gene therapy were noted. After 28 days, four of the six patients showed stable disease at the treated tumor site, whereas other tumor manifestations progressed. This study demonstrated that tomography-guided injection is suitable for performing intratumoral gene therapy.

Safety

In 1999 a clinical trial was conducted at the university of Pennsylvania in order to investigate gene transfer of the ornithine transcarbamylase (OTC) gene. An adenoviral vector containing the OTC gene was injected into adults suffering from a disease associated with OTC partial deficiency. After receiving the highest dose tested, an 18 year old man developed systemic inflammatory response and died a few days following treatment.

Following this tragedy, gene therapy trials raised many concerns in the press, the public, the scientific and medical communities and government agencies, although, in fact, there was no scientific reason to believe that the problems of concern are specific to gene therapy. In fact, results from the numerous trials conducted to date indicate that while the gene therapy approach has its limitations, these limitations are the exception rather than the rule [Sigel (2002) The Journal of Infectious Diseases 185:S52-S57] and that in some cases this approach is even safer and more effective than commonly practiced treatment approaches.

In the United States, gene therapy trials are conducted under guidelines similar to those practiced for other highly innovative biotechnological approaches. An FDA site-inspection random sampling of 15 % of active gene therapy clinical research applications, uncovered several sites at which areas for improvement were found and a few requiring regulatory or administrative action. However, the incidence of problems found was no greater than that seen in FDA inspections of efficacy trials described in applications for pharmaceutical agents.

An NIH report assessing the safety and toxicity of adenovirus (Ad)-based gene transfer concluded that "human gene transfer experiments using Ad-based vectors should continue-with caution" [Human Gene Therapy(2002) 13:3-13].

Testimonials

The following excerpts from Somia and Verma [(2000) Nature Reviews 1:91-99] clearly indicate that even researchers, which have in the past rejected

gene therapy concede that gene therapy can now be considered as a legitimate therapeutic approach.

With respect to vectors, Somia and Verma state that "Retroviral, lentiviral and adenoviral vectors do not seem to suffer from cytotoxic T cell responses. It could be that the vectors are completely replication-defective and that the incoming viral proteins do not elicit a cytotoxic T-cell response. Alternatively, the titers of at least the recombinant retro or lentiviral vectors tested so far might not be sufficiently high to elicit an immune response. Antibody response are also less of a concern, ...".

With respect to clinical efficacy and the promise of gene therapy Somia and Verma state that "The field of gene therapy has also a cause to celebrate. Alain Fischer and colleagues in Paris have successfully treated three babies (I-II months old), who suffer from a fatal form of severe combined immunodeficiency (SCID) syndrome [Cavazzana-Calvo M (2000) Science 288:669-672]."

"To all appearances the recipients are clinically cured, and the fantastic promise of gene therapy is realized."

"The use of modified MLV vectors and the extensive manipulation of the stem cells ... before transduction are a testimony to the continuous and incremental progress made in the field".

Somia and Verma also state that "Hemophilia (A and B) is another excellent model system for gene therapy....".

Thus, it is clear that Verma believes that following initial disappointments from gene therapy, as echoed in the publications cited by the Examiner, the field of gene therapy has overcome the problems of titers, delivery and safety.

Verma's faith in gene therapy is also echoed in reviews which cover current status of gene therapy in myocardial and angiogenic applications, see for example, Isner (2002) Myocardial gene therapy. Nature 415:234-239; High (2001) Gene therapy: a 2001 perspective. Hemophilia 7:23-27; and Hammond and McKirnan (2001) Angiogenic gene therapy for heart disease: a review of

animal studies and clinical trials. 49:561-567.

Another strong testimony to the effectiveness and safety of gene therapy comes from two studies conducted for several years in centers treating babies which have severe combined immunodeficiency (SCID), a lethal disease caused by mutation in genes involved in the immune response. More than ten babies with SCID were treated successfully by bone marrow cells gene transfer (Hacein-Bey-Abina et al N Eng J Med, 2002, Aiuti et al, Science 2002). Complete recovery was reported in all but one baby. Although one baby developed lympho proliferative disorder, which could be attributed to the use of retroviral vectors in gene transfer, the success rate of gene therapy in this otherwise lethal disease is impressive especially knowing that no other effective treatment is available for these SCID babies.

Cell therapy

Cellular transplantation for the treatment of chronic diseases has been developed significantly in the past few years. Examples include the use of myoblasts for treating Duchenne muscular dystrophy [Gussoni (1992) Nature 356:435-438], the use of fetal neural cells for treating Parkinson's disease [Freed (1992) N. Eng. J. Med. 327:1549-1555] and the use of fibroblasts over expressing factor VIII for treating hemophilia [Roth et al. (2001) N. Engl. J. Med. 344:1735-1742]. In the latter, successful combination of somatic cell transplantation with ex-vivo gene therapy in patients with severe hemophilia A is demonstrated. Briefly, an open-label phase I trial was conducted in six patients with severe hemophilia A. Dermal fibroblasts were electroporated with a plasmid containing sequences of the gene that encodes factor VIII. Stably transfected fibroblast clones that had incorporated the plasmid were selected on an antibiotics containing medium, isolated, expanded in non-selective medium and characterized for factor VIII expression, secretion, cell-growth and microbial safety. Cells (i.e., from a single clone) exhibiting the desired features were harvested one day prior to transplantation and administered to the recipient subjects. Patients were followed for 12 months following implantation of the genetically modified fibroblasts. As shown in

details in the paper, no adverse events related to the cellular implantation procedure were evident. Likewise, no long-term complications have developed and no inhibitors of factor VIII were detected. In four out of the six patients treated, plasma levels of active factor VIII rose above levels observed prior to implantation. The increase in plasma factor VIII activity coincided with a decrease in bleeding, a reduction in the use of exogenous factor VIII, or both (note patient 6). It will be appreciated that although factor VIII producing cells must be prepared for each patient individually, no technical obstacles underlie this procedure in general. Clinical changes lasted for at least 10 months, substantiating the feasibility of using genetically modified fibroblasts that produce factor VIII in patients with severe hemophilia A.

Preliminary experiments with cardiac tissue established that minced adult ventricular tissue could reorganize into a contractile mass when affixed to the apex of an injured heart [Bader (1978) J. Morphol. 155:349-357]. Subsequent studies in rats indicated that minced fetal atrial tissue could form stable, contractile grafts in ectopic skeletal muscle beds [Jockusch (1986) Proc. Natl. Acad. Sci. USA 83:7325-7329].

A series of breakthroughs in the last few years demonstrated that dispersed preparations of either skeletal myoblasts or cardiac myocytes were stable when engrafted onto donor mouse hearts and, in both cases, donor cells aligned with recipient cells [Koh (1993) J. Clin. Invest. 92:1548-1554; Soonpaa (1994) Science 264:98-101]. Moreover, in the fetal cardiomyocyte grafts, the formation of cell-to-cell contacts, complete with gap junction proteins, was documented [Koh (1995) J. Clin. Invest. 96:2034-2042]. More recent work has advanced these procedures to larger animal models, and the potential efficacy of cell grafting for myocardial repair in humans has been approached [Reinlib (2000) Circulation 101:e182-e187].

Thus, it can be concluded that although some researchers received gene therapy with mixed feeling, going as far as denouncing it as being ineffective and dangerous, numerous gene therapy trials in humans provided solid and irrefutable evidence that gene therapy is an effective and safe therapeutic

modality. Undoubtedly, current research efforts in this field will lead to additional successes and establishment of this field as a leading therapeutic approach.

In view of the above described clinical trials and reviews as well as personal statements made by scientists previously opposed to gene therapy, and further in view of the results provided by the Appendix enclosed herewith and described in a declaration signed by one of the inventors in this case, it is Applicant's strong opinion that the state of gene therapy at the time of filing of the instant application and the guidelines set forth in the instant application clearly indicate that the inventors had possession of the claimed invention at the time the application was filed.

Thus, it is Applicant strong opinion that the specification and the working examples provide guidance to use the claimed method to produce a therapeutic effect in an individual.

It is further the Applicant's opinion that the claimed invention is described in full, clear, concise, and exact terms that a skilled artisan would be able to practice the present invention without having to engage in undue experimentation.

In view of the above arguments, Applicant believes to have overcome the 35 U.S.C. § 112, first paragraph, rejections.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 23-37 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiners rejections are respectfully traversed. Claim 23 has now been amended.

With respect to claims 23-37 the Examiner points out that these claims are indefinite because in the claimed method of modifying the electrophysiological function of an excitable tissue region in an individual,

there is no conclusive statement which indicates that the desired effect has been achieved.

The Examiner further points out that claims 23-37 are indefinite in their recitation of "capable of", which is merely a potential property and not an actual property.

Claim 23 has now been amended to recite:

wherein each implanted cell forms:

(a) gap junctions with at least one cell of the excitable tissue region; and

(b) a functional ion channel or transporter;

thereby modifying the electrophysiological function of the excitable tissue region."

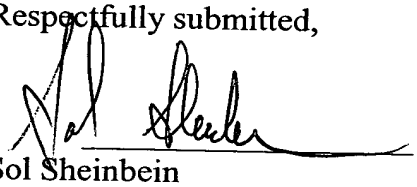
to thereby overcome the Examiner's rejection with respect to these claims.

With respect to claim 37, the Examiner points out a typographical error.

Claim 37 has now been amended to recite "functional gap junctions", to thereby overcome the Examiner's rejection with respect to this claim.

In view of the above amendments and remarks it is respectfully submitted that claims 23-37 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



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Date: February 27, 2003.

Encl.:
2-months extension fee;
Appendix;

Declaration by Dr. Yair Feld;
Articles by:
Siegel
High
NIH
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Somia
Herman
Morishita
Palmer
Cavazzana
Heon Chae
Peng
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Grines
Losordo
Hacein-Bey-Abina
Irani
Isner
Lubiatowski
Klatzman
Hickman
Kauczor
Freed
Gussoni
Koh 1995
Koh 1993
Soonpaa
Jokusch
Bader
Leslie
Lerche
Luo
Marom 1994(a)
Marom 1994(b)
Marom 1997

VERSION WITH MARKING TO SHOW CHANGES MADE

In the Claims:

23. (amended) A method of modifying the electrophysiological function of an excitable tissue region of an individual, the method comprising ~~the step of~~ implanting cells into the excitable tissue region, wherein each implanted cell ~~being~~forms:

(a) ~~capable of forming~~ gap junctions with at least one cell of the excitable tissue region; and

(b) ~~capable of forming~~ a functional ion channel or transporter, thereby ~~said functional ion channel or transporter being capable of~~ modifying the electrophysiological function of the excitable tissue region.

37. (amended) The method of claim 36, further comprising the step of expressing a second exogenous polypeptide in said at least a portion of said cells, said second exogenous polypeptide being capable of forming functional ~~pap~~ gap junctions within said at least a portion of said cells.

#7

APPENDIX

Brief description of the figures

FIGs. 1a-e are schematic illustrations of the multielectrode array (MEA) mapping technique. Figures a-b lustrate an MEA plate including 60 microelectrodes with an inter-electrode distance of 100 or 200 μm. Figures 1c-d are simultaneous extracellular recordings from all 60 electrodes and automatic calculations of local activation time (LAT), respectively. Figure 1e illustrates the construction of high resolution activation maps. Red and blue indicate activation at early and late sites, respectively.

FIGs. 2a-e are graphs showing voltage clamp analyses of Kv1.3 transfected fibroblasts (Figures 2a-b) and control non-transfected fibroblasts (Figure 2c). Figures 2d-e are low time resolution and high time resolution, respectively, of computer simulations comparing the rapid opening of the Kv1.3 channel with the typical cardiac sodium and total potassium currents.

FIGs. 3a-b are photomicropgraphs showing the distribution of Kv1.3 transfected fibroblasts (Figure 3a) and control non-transfected fibroblasts (Figure 3b) in a cardiomyocytes culture using vital fluorescent fast-DiO staining.

FIGs. 3c-d are confocal images of a control (Figure 3c) and Kv1.3transfected (Figure 3d) hybrid cultures co-stained with anti-MHC (green) and anti-Kv1.3 channel (red) antibodies. Note the presence of Kv1.3 channels in the transfected fibroblasts and absence of such channels in the cardiomyocytes and wild-type fibroblasts.

FIG. 4a is a photomicrograph illustrating the distribution of wild type fibroblasts in the hybrid culture using a fast DiO labeling.

FIGs. 4b-d are activation maps of non-transfected hybrid cultures generated prior to fibroblast seeding (Figure 4b), 5 days following fibroblast

seeding (Figure 4c) and following CTx application (Figure 4d). Note the uniform conduction in all cases.

FIG. 5a is a photomicrograph illustrating the distribution of Kv1.3 transfected fibroblasts in the hybrid culture using a fast DiO labeling.

FIGs. 5b-d are activation maps of Kv1.3 transfected hybrid cultures generated prior to fibroblast seeding (Figure 5b), 5 days following fibroblast seeding (Figure 5c) and following CTx application (Figure 5d). Note the appearance of conduction block (black arrows) with activation propagating (white arrow) around the clusters of fibroblasts responsible for the block and the resultant increase in total activation time to 95 ms.

FIG. 5e is an extracellular electrogram corresponding to a region which lacks fibroblasts (green electrode in Figure 5a). Electrograms were taken prior to (left), five days following fibroblast seeding (middle) and after CTx application (right).

FIG. 5f is an extracellular electrogram from an electrode positioned near a fibroblast cluster (yellow electrode in Figure 5a). Note the decrease in electrogram amplitude following fibroblast seeding (middle) and the return to baseline amplitude following CTx application (right).

FIG. 5g is an extracellular electrogram from an electrode (red electrode in Figure 5a) located in the area of conduction block (middle) and return to a single potential following CTx application (right).

FIGs 6a-g illustrate electrophysiological changes induced by fibroblast seeding. Figures 6a-b illustrate changes in electrogram amplitude at baseline (day 1) and following fibroblast seeding (days 2-6) in the control (Figure 6a) and study (Figure 6b) groups. Figures 6c-d illustrate changes in the normalized electrogram amplitude (normalized to the maximal amplitude value at each experiment) following CTx application in the control (Figure 6c) and study (Figure 6d) groups.

An asterik indicate $p<0.05$ when compared to baseline values. Figures 6e-f illustrate changes in the conduction block factor (CBF) at baseline (day 1) and following fibroblast seeding in the control (Figure 6e) and study (Figure 6f) groups. Note a significant ($*p<0.05$) gradual increase in CBF induced by the transfected fibroblasts (Figure 6f). Figure 6g illustrates changes in CBF following CTx application in the control (squares) and study (circle) groups. An asterik indicates $p<0.05$ when the CBF values in the study group were compared with the control group for each CTx concentration. A cross indicates $p<0.05$ when the CBF values following CTx application were compared with baseline values in the same group.

FIGs. 7a-b are photomicrograph depicting the distribution of Kv1.3 expressing fibroblasts in the AV node of a rat, using Fluorescent celltracker Fast DiI staining.

FIGs. 8a-c are ECG recordings of the left ventricular wall of a rat's heart transplanted with Kv1.3 transfected fibroblasts. Figure 7a is an ECG prior to transplantation. Figure 7b is an ECG 7 days following transplantation. Figure 7c is an ECG recording following Margatoxin application.

FIGs. 9a-c are ECG recordings of the AV node of a rat's heart transplanted with Kv1.3 transfected fibroblasts. Figure 7a is an ECG prior to transplantation. Figure 7b is an ECG 7 days following transplantation. Figure 7c is an ECG recording following Margatoxin application.

I. IN-VITRO ELECTROPHYSIOLOGICAL MODULATION OF CARDIOMYOCYTIC TISSUE BY Kv1.3 TRANSFECTED FIBROBLASTS

Experimental procedures

Preparation of cultured myocytes – As described in page 51 lines 13-18 of the instant application. Briefly, primary cultures of 1- to 2-day-old neonatal rat (Sprague Dawley) ventricular myocytes were prepared according to a modified

protocol previously described by Feld et al. [(2002) Circulation 105:522-529]. Briefly, following excision and dissection of the atria and great vessels, the ventricles were minced in phosphate buffer solution and later treated with RDB (IIBR, Ness Ziona, Israel). Minced tissue was centrifuged and dispersed cells were suspended in a culture medium (Ham's F10, Biological industries, Beit-Haemek, Israel) including 5% fetal calf serum, 5% horse serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 1 mM $CaCl_2$ and 50 mg/100 ml bromodeoxyuridine (BrdU, Sigma, B-5002). Cells were then cultured on a microelectrode array culture plate (at a density of 1.5 x 10^6 cells/ml). Fresh medium was replaced on alternating days.

Expression system and electrophysiological recordings – As described in pages 51-52 lines 19-3 of the instant application. Briefly, stable expression of Kv1.3 in NIH3T3 fibroblasts was effected by electroporating cells with pRC/CMV/Kv1.3 using a single pulse of 200 V, 960 μF delivered from a gene pulser transfection apparatus (Bio-Rad, Hercules, CA, USA). 48 hours following transfection, 400 μg/ml of G-418 (Gibco, LA, CA, USA) was added to select for cells exhibiting neomycin resistance. Two weeks later, colonies were picked and tested for channel expression. Whole-cell recordings were conducted at room temperature using Axopatch 200 (Axon Instruments, Whipple Road Union City, CA, USA). Data was collected using a Quadra 800 (Apple computers Cupertino, CA, USA) with PULSE software (HEKA Electronic), low-pass filtered at 5–10 kHz and sampled at 20 kHz. Electrodes for voltage-clamp experiments were made from fire-polished aluminum silicate glass, with a resistance of 5–6 MΩ. The pipette solution contained 140 mM KCl, 10 mM Na2ATP, 10 mM EGTA, 5 mM HEPES, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (pH 7.3). The bath solution contained: 140 mM NaCl, 3 mM KCl, 10 mM HEPES, 10 mM glucose, 2 mM $MgCl_2$ and 2 mM $CaCl_2$.

Preparation of fibroblast-cardiomyocyte co-cultures - As described in page 52 lines 4-14 of the instant application.

Multielectrode mapping - As described in pages 52-53 lines 19-9 of the instant application. Extracellular recordings from cultured cardiomyocytes were performed on a PC-based microelectrode data acquisition system (Multi Channel Systems, Reutlingen, Germany) including Multi-Electrode Array (MEA), pre- and filter-amplifiers, data acquisition board, and software. The MEA consists of a 50×50 mm glass substrate (Figure 1a), in the center of which is an embedded 0.7×0.7 or 1.4×1.4 mm matrix of 60 Titanium-nitride, gold contacts 30 μm diameter electrodes with inter-electrode distance of 100 or 200 μm. (Figure 1b) allowing simultaneously recording of extracellular potentials from all electrodes (Figure 1c). Data were recorded at 10-25 kHz with 12-bit precision. During the recording sessions, the MEA was constantly perfused with a gas mixture consisting of 5% CO_2 + 95% air. Temperature was maintained at 37.0 ± 0.1 °C.

Conduction block - As described in page 54 lines 1-14 of the instant application.

Fibroblast staining - As described in page 52 lines 15-18 of the instant application.

Immunohystochemistry – Hybrid cultures were grown on top of an MEA or on cover slips for 5 days prior to fixing in 4 % paraformaldehyde and permeabilization with 0.2% Triton X-100 (Sigma, Rehovot, Israel). Blocking was effected with 10 % goat serum (Biological industries, Beit Haemeq, Israel) for 1 hour at 37 °C. Immunostaining was effected using monoclonal rabbit anti-mouse Kv1.3 (Alomone labs, Jerusalem, Israel) and mouse anti-myosin heavy chain (anti-MHC, Chemicon, Temecula, CA, USA) antibodies in blocking buffer for 24 hours at 4 °C. Preparations were then incubated with FITC-conjugated anti-mouse IgG and Rhodamine-conjugated anti-rabbit IgG secondary antibodies (both from Chemicon) for 1 hour. Samples were visualized using an inverted fluorescent

microscope (Zeiss, Axiovert 135 Oberkochen, Germany) or using a confocal microscope [Nikon Eclipse E600 microscope and Bio-Rad Radiance 2000 scanning system Hercules, CA, USA at a magnification of X 60 and a z resolution of 0.5 μm]

Results

Voltage clamp analysis of the transfected fibroblasts – NIH 3T3 fibroblasts were transfected with the Kv1.4 potassium channel and voltage clamp analysis was effected.

As shown in Figures 2a-c, voltage clamp analysis (Figure 2a) and the corresponding reconstructed current-voltage (I-V) curve (Figure 2b) demonstrated the presence of a high-magnitude outward potassium current with rapid activation kinetics in the channel transfected fibroblasts. Time to peak current at a voltage where the channel is fully activated was in the order of 4-5 ms. In contrast, no significant transmembrane currents were observed in non transfected fibroblasts (Figure 2c). The unique properties of the Kv1.3 channel [Marom S, Levitan IB. State-dependent inactivation of the Kv3 potassium channel. Biophys. J. (1994) 67:579-589; Marom S. Slow changes in the availability of voltage-gated ion channels: effects on the dynamics of excitable membranes. J. Membr. Biol. (1998) 161:105-113; Marom S, Abbott LF. Modeling state-dependent inactivation of membrane currents. Biophys. J. 1994;67:515-520] were also exhibited in Figures 2d-e in which a computer simulation compared the rapid opening of the Kv1.3 channel to that of typical cardiac sodium and total potassium currents [Luo CH, Rudy Y. A model of the ventricular cardiac action potential. Depolarization, repolarization, and their interaction. Circ. Res. (1991) 68:1501-1526].

Thus, the hereinabove voltage clamp results demonstrated some of the possible advantages of using the Kv1.3 channel in the present methodology. First, the relatively rapid activation and relatively slow kinetics from open to close states may allow maximal modulating effects on the neighboring cells during various

phases of the action potential. Second, the robust expression of the potassium channel within the fibroblasts generated a very high-magnitude outward potassium current following membrane depolarization. Third, the high affinity of the Kv1.3 channels to the inhibitor CTx allowed the usage of low dosages (not affecting the cardiomyocytic tissue) in the co-culture studies.

Structural analysis of hybrid cultures - The presence of ectopic fibroblasts within cardiomyocyte-fibroblast co-cultures was assessed during the experiments using vital Fast-DiO staining. Figure 3a displays a typical fibroblast distribution in a control culture, while Figure 3b presents a similar distribution in co-cultures including Kv1.3 transfected fibroblasts. The ability to accurately locate the fibroblasts with relevance to the microelectrode array enabled the accurate assessment of their possible local electrophysiological effects. These results were substantiated by co-staining experiments using anti-MHC and anti-Kv1.3 antibodies (Figures 3c-d), which confirmed the presence of fibroblasts expressing Kv1.3 channels in the study group and absence of these channels in cardiomyocytes and in non-transfected fibroblasts.

Electrophysiological characterization of hybrid cultures -MEA recordings obtained during culture development demonstrated, in both groups, frequent changes in the rate and position of the firing focus. Generally, the site of spontaneous firing was outside the mapping area and changes in "pace-maker" position were identified by shifts in the earliest activation sites within the MEA.

Figure 4a depicts typical clustered fibroblast-seeding pattern in the control group lacking transfection. Figures 4b-c show activation maps obtained prior to- and 5 days following fibroblast seeding, respectively. In all cases a uniform electrophysiological conduction was observed. Interestingly, following application of CTx, a specific Kv1.3 blocker, no significant changes in conduction properties were observed, suggesting that wild-type fibroblasts do not express the channel endogenously.

Hybrid cultures containing Kv1.3 transfected fibroblasts developed several areas of significant conduction delays and conduction blocks. Figure 5a shows a typical fibroblast-seeding pattern in the study group. Figure 5b depicts an activation map prior to cell grafting with a uniform conduction. Fig. 5c shows an activation map of the same culture 5 days following grafting of the transfected fibroblasts. Note the appearance of a conduction block (black arrows) identified by the significant time delay on electrodes on opposite sides of the block and by the change in wavefront orientation circumventing the fibroblast clusters (white arrows).

The presence of conduction block was substantiated by the significant increase in activation time from a baseline value of 40 to 95 ms. These conduction changes were fully reversed following administration of CTx (Figure 5d) with resumption of uniform conduction and recovery of total activation time of 45 ms.

The localized electrophysiological effects of the transfected fibroblasts can also be appreciated by changes in the morphology of the recorded electrograms. Electrogram amplitudes did not change significantly from baseline in electrodes located away from the fibroblasts clusters (Figure 5e) but were reduced significantly in electrodes located near transfected fibroblast clusters (Figure 5f) and were associated with double potentials in areas of conduction blocks (Figure 5g). As shown in Figures 5f-g, electophisilogical properties of Kv1.3 expressing hybrid culture could be reversed following CTx application (right part of the graphs).

Signal amplitude – As shown in Figure 6a, the presence of non-transfected fibroblasts in the co-culture did not reduce rather increased the amplitude of the recorded electrograms during culture maturation. In contrast, seeding of Kv1.3 transfected fibroblasts generated a significant reduction in the peak-to-peak electrogram amplitude by 47, 55, 66, and 79 % from its baseline value at 2, 3, 4 and 5 days post fibroblast grafting, respectively($p<0.05$, Figure 6b). Consequently,

mean electrogram amplitude was significantly lower in the study group as early as day 2 post fibroblast seeding. CTx application did not change electrogram amplitude in the control group (Figure 6c) but had a dose-related effect in the study group (Figure 6d) with a 23% increase in the normalized electrogram amplitude at a CTx concentration of 100 nM ($p<0.05$).

Conduction blocks - A conduction block factor (CBF) was defined to evaluate the number of electrodes associated with significant conduction delays, essentially, more than 25% of the total MEA activation time. The average delay time in these electrodes was 25.8±0.1 ms. Figures 6e-f depict changes in CBF at baseline (day 1) and on consecutive days following fibroblast seeding. As shown in Figure 6e, CBF did not change markedly and was minimal during the recording period in the control group. In contrast, in the study group, CBF increased gradually from a baseline value of 0.8 ± 0.8 up to 6.7 ± 1.8, 6 days following fibroblast seeding ($P<0.05$, Figure 6f).

CTx application did not change the CBF in the control group but had a dose-related effect in the study group with a reduction of CBF from an initial value of 6.0 ± 1.1 to 2.0 ± 0.8 at a CTx dose of 0 and 100 nM, respectively (Figure 6g). In addition, significant differences were noted in CBF values between the control and the study group prior to CTx application and at 1 and 10 nM ($P<0.05$). Application of 100 nM of CTx restored CBF in the study group to a value not significantly different than that of the control group.

II. IN-VIVO ELECTROPHYSIOLOGICAL MODULATION OF CARDIOMYOCYTIC TISSUE BY KV1.3 TRANSFECTED FIBROBLASTS

Experimental Procedures

Tissue-transplanted fibroblast staining - In order to follow the distribution of transplanted cells within the rat hearts, fibroblasts were labeled with a fluorescent lipophilic tracer; Fast DiI (Molecular probes, Eugene, OR USA)

diluted at 1:500 with low ionic strength solution for five minutes prior to seeding in the cultured cardiomyocytes.

Cell transplantation - NIH 3T3 fibroblasts transfected with Kv1.3 channel (n = 5) or control untransfected cells (n = 2) were injected to the left ventricular freewall or to the atrio-ventricular junction (AV node) of rats (Sprague Dawley). About 1-3 million cells were injected at each operation (left thoracotomy).

Each rat was stimulated to find the Wenckebach frequency, and the effective refractory period, prior to cell transplantation and 5-7 days following the procedure.

In vivo stimulation and electrophysiological recording - Rats were anesthetized with Ketamine 120 mg/kg and Xylazine 17 mg/kg. Left thoracotomy was performed, and electrodes were attached to the left ventricle wall. The refractory period of rat myocardium was tested using an ECG recording of the response to multistep stimulation. The rat heart was stimulated with bipolar tungsten electrodes according to a fixed protocol of 20 stimuli of 150 microampere every 250 ms, followed by a single stimulus of the same current.

Results

Atrio-ventricular electrophysiological modification by Kv1.3 expressing fibroblasts - The average RP of the hearts was 104 ± 8 ms prior to transplantation and 166 ± 22 ms following transplantation with Kv1.3 expressing fibroblasts (P = 0.01). As anticipated by results obtained in-vitro, upon application of margatoxin, a specific kv1.3 channel blocker, the RP has fallen back to 130±10 ms in the transfected group (P=0.038), and showed no change in the non transfected one (base line: 95 ± 7 ms, following transplantation: 95 ± 7 ms, and following margatoxin application: 97.5 ± 4 ms). Figures 8a-c show ECG recordings from a representative experiment in which transfected fibroblasts were injected to the left ventricular wall. As shown, prior to Kv1.3 expressing fibroblast transplantation the refractory period was 95 ms (Figure 8a). 7 days following transplantation of

the transfected fibroblasts the refractory period was extended to 170 ms (Figure 8b), while following Margatoxin application (10 μg/Kg) the refractory period almost completely returned to a baseline of 110 ms (Figure 8c), substantiating that the electrophysiological modulation of the cardiac tissue is Kv1.3 mediated.

Kv1.3 transfected fibroblasts were also transplanted to the AV node or rat's heart and results of one representative experiment are shown in Figures 9a-c. Here too, the refractory period prior to transplantation was 140 ms (Figure 9a), and 220 ms 7 days following transplantation (Figure 9b). Margatoxin application has reduced the refractory period to 150 ms (Figure 9c), illustrating the reversible properties of the electrophysiological modulation, generated according to the teachings of the present invention. Figures 7a-b are photomicrograph depicting the distribution of Kv1.3 expressing fibroblasts in the AV node of a rat, using Fluorescent celltracker Fast DiI staining, substantiating that the cellular implants integrated in the affected tissue and survived following transplantation.